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Physiologically Based Pharmacokinetic Modeling of the Pregnant Rat: A Multiroute Exposure Model for Trichloroethylene and Its Metabolite, Trichloroacetic Acid

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Physiologically Based Pharmacokinetic Modeling of the Pregnant Rat: A Multiroute Exposure Model for Trichloroethylene and its Metabolite, Trichloroacetic Acid. FISHER, J. W., WHITTAKER, T. A., TAYLOR, D. H., CLEWELL, H. J. III, AND ANDERSEN, M. E. (1989). *Toxicol. Appl. Pharmacol.*, **99**, 395–414. A physiologically based pharmacokinetic (PB–PK) model was developed to describe trichloroethylene (TCE) kinetics in the pregnant rat exposed to TCE by inhalation, by bolus gavage, or by oral ingestion in drinking water. The kinetics of trichloroacetic acid (TCA), an oxidative metabolite of TCE, were described by a classical one-compartment pharmacokinetic model. Among the required model parameters for TCE, partition coefficients (PCs) and kinetic constants for oxidation were determined by vial equilibration and gas uptake methods, respectively. The fat:blood PC was 33.9; the blood:air PC was 13.2; and the fetal tissue:fetal blood PC was 0.51. TCE was readily metabolized with high substrate affinity. In naive and pregnant female rats the maximum velocities of oxidative metabolism were 10.98 ± 0.155 and 9.18 ± 0.078 mg/kg/hr, while the estimated Michaelis constant for the two groups of rats was very low, 0.25 mg/liter. The first-order rate constant for oral absorption of TCE from water was 5.4 ± 0.42/hr^{-1} in naive rats. With TCA, the volume of distribution (0.618 liter/kg) and the plasma elimination rate constant (0.045 ± 0.0024/hour) were estimated both from intravenous dosing studies with TCA and from an inhalation study with TCE. By comparison of the two routes of administration, the stoichiometric yield of TCA from TCE was estimated to be 0.12 in pregnant rats. To develop a data base for testing the fidelity of the PB–PK model, inhalation and bolus gavage exposures were conducted from Day 3 to Day 21 of pregnancy and a drinking water exposure from Day 3 to Day 22 of pregnancy. Inhalation exposures with TCE vapor were 4 hr/day at 618 ppm. The TCE concentration in drinking water was 350 μg/ml and the gavaged rats received single daily doses of 2.3 mg TCE/kg. Time varying physiological parameters for compartment volumes and blood flows during pregnancy were obtained from the published literature. Using the kinetic parameters determined by experimentation, TCE concentrations in maternal and fetal blood and TCA concentrations in maternal and fetal plasma were predicted from the PB–PK model by computer simulation and compared favorably with limited data obtained at restricted time points during pregnancy for all three routes of exposure. On the basis of the PB–PK model, fetal exposure to TCE, as area-under-the-curve, ranged from 67 to 76% of maternal exposure. For TCA the fetal exposure was 63 to 64% of the maternal exposure. The fetus is clearly at risk both to parent TCE and its TCA metabolite. With further validation, PB–PK modeling of expected fetal exposure should prove helpful in the design and interpretation of teratology and reproductive toxicology studies with a variety of volatile chemicals.

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Trichloroethylene (TCE), a widespread environmental contaminant, has been listed by the U.S. Environmental Protection Agency as a "possible human carcinogen" (U.S. EPA, 1987) and its oxidative metabolite, trichloroacetic acid (TCA), is a recognized carcinogen in rodents (Elcombe, 1985; Herren-Freud *et al.*, 1987). In addition to cancer, a large number of other toxic endpoints have been examined with this important commercial solvent (U.S. EPA, 1985, 1987). Teratogenic studies with rats have either been negative (Beliles *et al.*, 1980) or demonstrated only slight developmental delays (Dormueller *et al.*, 1979). In contrast, Taylor *et al.* (1985) observed irreversible deficits in locomotor activity in rat pups born to dams that had been exposed to TCE in drinking water during pregnancy. Rat pups exposed to TCE *in utero* also exhibited reduced brain uptake of 2-deoxyglucose (Noland-Gerbec *et al.*, 1986). These studies clearly indicated substantial effects of TCE or its metabolites on the developing fetus. By themselves, however, these studies do not suffice to estimate fetal exposure in these particular dosing situations. Only the maternal dose regimens were specified and no measurements of fetal concentrations of TCE and/or its metabolites were obtained. In an effort to more accurately predict fetal dosimetry, we began development of generic pharmacokinetic models for the pregnant and the lactating rat (Fisher *et al.*, 1987) and applied these models to the study of TCE and TCA, its stable, persistent oxidative metabolite.

A complication encountered in pharmacokinetic modeling in pregnancy is the time-dependence of compartment volumes, blood flows and metabolic parameters at various stages of pregnancy. Most of the recent physiologically based pharmacokinetic (PB–PK) models developed for other volatile chemicals have been for animals where these physiological parameters were considered to be constant over time. In constructing a PB–PK model for TCE in pregnancy, the increase in maternal body weight, in fetal and placental weight, and in blood flows to various regions during pregnancy can be found in the physiological literature. Other model constants, related to tissue partitioning and rates of metabolism of TCE, have to be measured directly by suitable experimentation.

In the present work we have developed a PB–PK structure for TCE in pregnancy which has seven physiological compartments, including placenta, fetus, and mammary tissue. In addition, TCA kinetics were linked to TCE oxidation and were modeled by a hybrid approach which included the dam as a single compartment for TCA (National Research Council, 1986). Blood flow carried TCA to the placenta where the TCA diffused into the fetal circulation. The overall model was developed from literature for physiological variables and from appropriate metabolic experiments in naive and pregnant female rats. The model was then used to predict maternal and fetal concentrations of TCE and TCA expected following three types of repeated exposures with the pregnant rats. These exposures were inhalation of 618 ppm TCE, ingestion of drinking water containing TCE, and daily single dose gavage with water containing TCE. The adequacy of the modeling approach to predict TCE and TCA disposition during pregnancy was inferred from the correspondence between prediction and observed concentrations in limited numbers of observations from pregnant rats and fetuses killed at restricted times during gestation.

METHODS

Animals. Female, cesarean derived Fischer-344 rats (170–200 g), obtained from Charles River Breeding Laboratory (Kingston, NY) were used for kinetic constant determinations. Pregnancy was initially determined by vaginal smears and by observing for the presence of sperm. Timed-pregnant female cesarean derived Fischer-344 rats were delivered to the laboratory from Harlan Sprague Dawley (Indianapolis, IN) on Day 3 of pregnancy. These rats were used for the acute inhalation exposure and the subchronic exposures. All rats were kept in separate cages and allowed access to commercial rat chow (Purina rat chow) and water *ad libitum*.

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Chemicals. TCE (>99%) and TCA (98%) were obtained from Aldrich Chemical Co. A TCA working stock for gas chromatographic standards, prepared by dissolving TCA in physiological saline prior to experimentation, was maintained as a working stock for 2 weeks. TCE stock solutions for gas chromatographic standards were not stored, but were prepared immediately prior to experimentation.

TCE and TCA analyses. Heparinized blood samples for TCE analysis (0.1 ml) were dispensed into 1.5-ml glass Teflon-coated septa screw cap vials containing 1.0 ml of *n*-hexane. These vials were shaken for 1 hr on a Buchler Vortex evaporator at ambient temperature (23–25°C) before gas chromatographic analysis of the TCE in *n*-hexane.

A Model 5890A Hewlett–Packard gas chromatograph equipped with a polar DB-17 30 m capillary column (0.25 mm i.d.), an electron capture detector, and an automatic sampler was used for TCE analysis. The oven temperature was 70°C, injector temperature 125°C, and detector temperature 300°C. The column split ratio was five and the argon/methane carrier flow was 0.50 ml/min through the column. Extraction efficiency of TCE into *n*-hexane from maternal and fetal blood was greater than 93%. The limit of detection for TCE was 0.03 μg/ml and the retention time was 3.4 min.

Heparinized maternal and fetal blood samples (0.2 ml each) for TCA analysis were placed in capillary blood serum separators and centrifuged for 0.5–1.0 min in a Brinkman 3200 microcentrifuge. The supernatant (plasma) was collected and either frozen or prepared for immediate gas chromatographic analysis. Frozen samples were analyzed within 5 days. The TCA assay procedure included a methylation step in order to use gas chromatography for detection. Fifty microliters of either fresh or thawed plasma was placed in a 1.5 glass vial containing 0.1 ml of chilled 3N methanolic HCl and then 1.0 ml of *n*-hexane was added. These samples were incubated for 30 min at 100°C prior to gas chromatographic analysis of the TCA methyl ester in *n*-hexane. Gas chromatographic conditions for TCA methyl ester analysis were the same as for TCE analysis except that the oven temperature was 100°C. Extraction of TCA methyl ester from the fetal and maternal plasma into *n*-hexane was greater than 91%. The limit of detection for the TCA methyl ester was 0.07 μg/ml and the retention time was 6.5 min.

TCA kinetic constants by IV dosing. Four cannulated rats (Day 14–15 of pregnancy) were given 4.0 mg TCA/kg in saline intravenously. Blood samples were collected from jugular cannulae at 1, 3, 10, 22, 28, and 47 hr postexposure. The plasma TCA elimination rate constant for the pregnant rat was calculated from log-linear regression of the TCA plasma concentration over time. Volume of distribution (liter/kg) was estimated by dividing the dose (mg/kg) of TCA by the initial TCA plasma concentration (liter; Y intercept at time zero).

Single 4-hr TCE inhalation exposure. A single 4-hr inhalation exposure to 600.4 ppm TCE (time weighted average, TWA) was conducted with six jugular cannulated Day 12 pregnant rats. The inhalation chamber, a six compartment plexiglas container, and the cannulation procedure have been described by McDougal *et al.* (1985). Atmospheric levels of TCE in the chamber were monitored every 5 min with a 5880 Hewlett–Packard gas chromatograph equipped with a 6-ft $\frac{1}{8}$-in.-i.d. column containing 3% SE 30 on 80/100 mesh Supelcoport and an injector temperature of 200°C, oven temperature of 100°C, and FID detector temperature of 250°C. The TCE retention time was 0.84 min. Blood samples (about 0.3 ml) were collected from the cannulae using 1.0 heparinized syringes at 0.083, 0.50, 1.0, 1.5, and 2.0 hr postexposure for TCE and TCA analyses as described earlier. Additional samples (0.2 ml) were taken at 5.0, 21.0, 29.0, and 46.0 hr postexposure for TCA analysis. This exposure permitted calculation of the yield of TCA from TCE oxidation.

Subchronic inhalation exposure: Seven pregnant rats (Day 3 of pregnancy) were exposed to 618 ppm TCE (TWA) in a 31 liter battery jar inhalation exposure system (Leach, 1963; Andersen *et al.*, 1984) 4 hr/day, 5 days/week for 3 weeks. In each exposure, atmospheric concentrations of TCE were analyzed every 5 min by a gas chromatograph (Model 5880A, Hewlett Packard) equipped with a flame ionization detector and an automatic sampling valve. A 10-ft $\frac{1}{8}$-in.-o.d. stainless column was used containing 3% SE 30 on 80/100 mesh Supelcoport. The injection temperature was 125°C, the detector temperature, 300°C, and the oven temperature, 80°C. The retention time for the TCE was 2.3 min. On Day 20 of pregnancy, maternal and fetal blood was collected for TCA analysis from three rats exposed to TCE on the previous day (20 hr postexposure). TCE and TCA analyses were also conducted on blood samples collected from four rats immediately after the 4-hr exposure. Pregnant rats were killed by cervical dislocation immediately after removal from the exposure chamber. The abdomen was opened and 0.3 ml of maternal blood was collected with a heparinized syringe from the inferior vena cava. Concurrently, fetuses were decapitated and a total volume of 0.3 ml of fetal blood was obtained with heparinized capillary tubes from three to five fetuses. This exposure was used to estimate the transfer coefficients for TCA across the placenta.

Subchronic drinking water exposure. Ten pregnant rats (Day 3 of pregnancy) were provided drinking water containing 350.0 ± 9.5 (SE) μg of TCE/ml of water, 5 days per week for 3 weeks. Five pregnant rats received distilled water and served as controls. Fresh TCE–water solutions (three 2 liter flasks) were prepared daily, by adding 1.0 ml of pure TCE to 2 liters of distilled water in a stoppered flask and stirring overnight. The TCE–water solutions from the flasks were combined in a 5 liter glass jar, stirred again for 1 hr, and dispensed into a 125-ml amber glass drinking water bottles, then capped with a nalgene stopper containing a sipping tube.

The initial TCE concentration in a portion of the water bottles was measured daily and averaged over 3 weeks. To determine the loss of TCE from the drinking water, the same drinking water bottles were sampled again 24 hr later. The average loss of TCE from the drinking water bottles in the 24-hr period, on the basis of weekly averages, was slightly greater than 50% of the initial TCE concentration. TCE-water consumption was tabulated each day yielding an average consumption of 14.5 ml/day/rat for a 3-week period. Control rats drank an average of 16.9 ml distilled water/day/rat for the same 3-week period.

In one case maternal blood was collected from four rats as early as Day 18 of pregnancy (1000–1100 hours) for TCA analysis. On Day 21 of pregnancy (1000–1100 hr) maternal and fetal blood were collected for TCE and TCA analyses from six rats.

Subchronic gavage exposure. Nine pregnant rats (Day 3 of pregnancy) were dosed by bolus gavage with 2.3 mg TCE/kg in water (0.8 to 1.5 ml) 5 days/week for 3 weeks. Three pregnant rats were gavaged with distilled water and served as controls. Gavage animals were dosed with the TCE–water mixture used in the drinking water study. All aliquots of the TCE–water mixture were analyzed to quantify the amount of TCE in the water. On Day 20 of pregnancy, maternal and fetal blood was collected from four rats killed immediately after gavage for TCE and TCA analyses. Three hours after gavage another five rats were killed for TCA analysis. Drinking water and gavage animals were killed and blood was collected in the same manner as with the inhalation animals.

Gas uptake. Gas uptake techniques have been used to assess *in vivo* kinetic constants for metabolism (Filser and Bolt, 1979; Andersen *et al.*, 1980; Gargas *et al.*, 1986a,b). Atmospheric concentrations of TCE in the gas uptake chamber were monitored by a gas chromatograph (Model 5890A, Hewlett Packard) equipped with an automatic sampling valve, flame ionization detector and a 10-ft $\frac{1}{8}$-in.-o.d. stainless steel column (3% SE30 on 80/100 mesh Supelcoport). Injection temperature was 125°C, detector temperature, 300°C, and oven temperature, 100°C. Atmospheric TCE samples were taken at 5 min after injection of TCE into the chamber atmosphere and then every 10 min for the duration of exposure (1.5–6.0 hr). The retention time for TCE was 2.5 min.

Experimental exposures for kinetic constant determinations were conducted with nonpregnant, and Day 13–14 pregnant rats. Nonpregnant and pregnant rats (four per concentration) were exposed to initial TCE concentrations of 5075, 2200, 1100, and 111 ppm; and 2050, 1005, and 103 ppm respectively. V_{max}, the maximum rate of metabolism (mg/hr) for a 1.0-kg animal (allometrically scaled) was estimated by computerized nonlinear least-squares techniques (Simusolv, Dow Chemical Co.). The pregnancy model (Fig. 1; Appendix II) for Day 14 of pregnancy was used to obtain statistical best fit estimates of V_{max}, while the four-compartment model structure (Ramsey and Andersen, 1984) was used for fitting V_{max} with the naive rats. Physiologic parameters for the pregnancy model (Fig. 1) are discussed later under Methods (Physiological parameters) and reported in Table 1, while TCE kinetic constants are reported in Table 2. Appendices I and II contain pregnancy model nomenclature and mathematical equations.

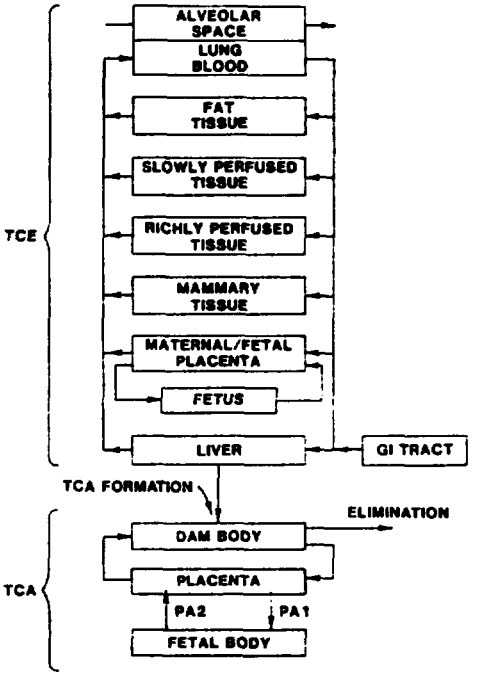
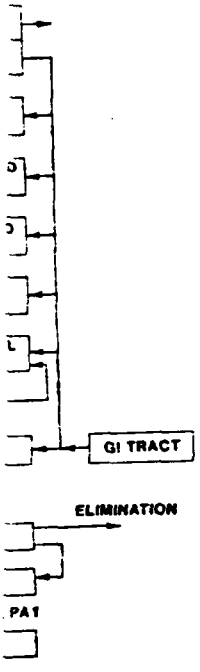


FIG. 1. Physiologically based pharmacokinetic pregnancy model used to describe the disposition of trichloroethylene (TCE) and the compartmental model used to describe the disposition of trichloroacetic acid (TCA). TCE enters the body by inhalation or by oral ingestion (gavage or drinking water). TCA is formed from metabolism of TCE in the liver. Details of the pregnancy model are found in the Appendices.

First-order oral uptake rate constant for TCE. Three adult naive rats were prepared with jugular cannulas and gavaged with 7.6 mg TCE/kg in a total volume of 4 ml of water. Heparinized blood samples were collected at 2, 5, 10, 20, and 30 min post-gavage. The TCE-water solution for the oral uptake study was prepared by mixing TCE and distilled water overnight in a 25-ml Teflon sealed glass vial with a stirbar and magnetic mixer. The first-order rate constant (K_a) for oral absorption TCE was determined by using Simusolv to optimize the naive rat model within the constraints of the model. This calculated K_a value for TCE was then used to describe repeated gavage dosing of the pregnant rats.

Partition coefficients. TCE tissue/air partition coefficients were determined for blood, muscle, fat, and liver



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using virgin females and for blood, placenta, fetal blood, and fetal tissue using near term pregnant females. The TCE mammary tissue/air partition coefficient was determined using an early postpartum lactating rat. The vial-equilibration method (Sato and Nakajima, 1979; Gargas *et al.*, 1989) was employed to measure tissue solubilities because of its simplicity and the large existing data base for other volatile organics developed using this method (Gargas *et al.*, 1989). Tissue/air TCE partition coefficients were divided by the blood/air partition coefficient to obtain the tissue/blood partition coefficient. A Perkin-Elmer 3920 gas chromatograph equipped with a 6-ft $\frac{1}{8}$-in.-o.d. stainless steel column containing 3% SP 2100 was used for TCE analyses. The oven temperature was 100°C, the injector temperature 175°C, and the detector, 250°C. The retention time for TCE under these conditions was 0.34 min.

TCA is a nonvolatile chemical unsuited for vial equilibration methods. Tissue/saline TCA partition coefficients were determined for blood and placenta using a centrifugation technique (Jepson, 1986). The placenta/saline TCA partition coefficient was divided by the blood/saline partition coefficient to estimate the placenta/blood partition coefficient.

Physiological parameters. Certain time dependent physiological changes that occur during pregnancy (Table 1) were incorporated into the pregnancy model by using the table function of the computer simulation software, Advanced Continuous Simulation Language (ACSL) (Mitchell and Gauthier, 1981). All tissues were assumed to have unit density. Physiologic constants that vary with time included dam body weight gain and growth of the placenta, fetus, mammary, and fat tissues. Accompanying increases in maternal blood supply to the placenta and mammary tissues and fetal blood supply to the placenta tissue were also taken into account. Fetal growth from Days 14 to 22 of gestation was described by

$$\text{FW (fetal weight, grams)} = [\alpha(t - 13)]^3, \quad (1)$$

where α is a specific growth constant (0.17) and t is gestation time in days (Huggett and Widdas, 1951). Fetal weight before Day 13 was considered to be insignificant. At birth, pup weight (3.5–4.1 g) agreed favorably with the predicted pup weight of 3.6 g. The $(t - 11)$ time function reported by these authors was adjusted to $(t - 13)$ to account for an increase in gestation time of 2 days for the rats used in this study.

Placental growth from Days 14 to 22 of gestation was described by

$$V_{pla}\text{ (placenta)} = 0.102 \exp[0.217(t - 13)]\text{ (ml)}, \quad (2)$$

where t is the gestation time in days (Olanoff and Anderson, 1980). Placenta weights (detached disks, 0.45 to 0.50 g) on Day 20 of pregnancy compared favorably to the predicted weight.

Maternal blood flow to the placenta on Days 14 to 22 of gestation was described as a function of placenta weight as shown by

Q_{pla} (maternal placental blood flow)

$$= V_{pla} \cdot 75.0\text{ (ml/hr)}. \quad (3)$$

where 75.0 is the placenta flow rate constant (ml/hr/g) (Olanoff and Anderson, 1980).

Fetal blood flow to the placenta (Q_{fet}) on Days 14 to 22 of gestation was 25% of the maternal blood flow to the placenta (Eq. 3). This estimate of fetal blood flow was derived from the pregnancy model developed by Olanoff and Anderson (1980).

The fetal growth (Eq. 1), placental growth (Eq. 2) and maternal blood flow to the placenta (Eq. 3) were calculated for each day of gestation (Days 14–22) and multiplied by a factor of 7.0 to reflect the observed average litter size for these studies. Placenta weight (volume) was expressed as a percentage of maternal body weight and ranged from 0.4% on Day 14 of gestation to 2.9% of body weight on Day 22 of gestation. The calculated fetal litter weight for seven fetuses ranged from 0.034 g on Day 14 of gestation to 25.1 g on Day 22 of gestation. Fetal weight and was not scaled to maternal body weight. The effect of litter size on TCE and TCA kinetic profiles in the dam and fetus is discussed under Discussion. Maternal blood flow to the placenta (for the litter) was expressed as a percentage of cardiac output and ranged from 1.5% of cardiac output on Day 14 of gestation to 9.9% on Day 22 of gestation. Fetal blood flow (for the litter) to the placenta was expressed as a percentage (25%) of maternal placental blood flow and ranged from 0.37% of maternal cardiac output on Day 14 of gestation to 2.5% on Day 22 of gestation.

Mammary tissue was assumed to reach 21% of its total growth by Day 10 of pregnancy and 59% by Day 20 of pregnancy (Griffith and Turner, 1961). Mammary tissue weight, expressed as a percentage of body weight, was assigned values of 2, 2.7, 3.2, and 4.4% at Days 3, 12, 14, and 22 of pregnancy (Knight *et al.*, 1984). Maternal blood flow to the mammary tissue was described as a linear increase (1 to 9% of cardiac output) from Day 3 to Day 22 of gestation (Hanwell and Linzell, 1973).

Fat accumulation during pregnancy was described according to Naismith *et al.* (1982). Body fat values, expressed as a percentage of body weight, were 6, 6, 8, and 12% on Days 3, 9, 16, and 22 of gestation, respectively.

Maternal weight gain during pregnancy was estimated by subtracting the calculated placental and fetal weights from the measured weight of the pregnant rat. Maternal weight gain was described as a linear function increasing by 10% of the initial weight (Day 3 of gestation) by Day 22 of gestation. Volume of the liver, slowly perfused and richly perfused tissue groups were given values of 4%, 70.6 to 59.7%, and 8%, of body weight. Blood flow to the liver, fat, slowly perfused and richly perfused tissue groups were respectively 25, 9, 15, and 48.1 to 29.6% of cardiac output.

TABLE I

PHYSIOLOGICAL CONSTANTS USED IN THE PB-PK MODELS FOR THE NAIVE AND PREGNANT RAT

	Naive dam	Pregnant dam
Body weights (kg)		
Acute inhalation	—	0.192
Subchronic inhalation	—	0.187–0.206[a]
Single gavage	0.170	—
Subchronic gavage	—	0.144–0.158[a]
Subchronic drinking water	—	0.168–0.185[a]
	Percentage of body weight	
Liver	4.0	4.0
Richly perfused	5.0	4.0
Slowly perfused	76.0	70.6–59.7
Fat	6.0	6.0–12.0
Mammary tissue	—	2.0–4.4
Maternal/fetal placenta[b]	—	0.4–2.9
Fetal litter[b]	—	0.034–25.1g
Flows (liter/hr)		
Alveolar ventilation	14.0·body $wt^{0.74}$	19.9·body $wt^{0.74}$
Cardiac output	14.0·body $wt^{0.74}$	14.0·body $wt^{0.74}$
	Percentage of cardiac output	
Liver	25.0	25.0
Richly perfused	51.0	48.1–29.6
Slowly perfused	15.0	15.0
Fat	9.0	9.0
Mammary tissue	—	1.0–9.0
Maternal placenta[b]	—	1.5–9.9
Fetal placenta[b]	—	0.37–2.5

[a] Measured initial body weight on Day 3 of pregnancy and predicted maternal body weight on Day 22 of gestation (less fetuses and placentas).

[b] Litter size equals seven.

RESULTS

Partition coefficients. The blood:air partition coefficient (PC) was lower in the pregnant rat (13.2 ± 0.34) than in the naive female rat (15.0 ± 0.35). It was lower still for fetal blood (9.6 ± 0.33). TCE partitioned into tissues as expected for a chemical that is moderately lipophilic (Table 2).

Rates of metabolism. When estimating the kinetic constants from the gas uptake experiments (Figs. 2A and 2B), both V_{max} and K_m for TCE metabolism were initially allowed to vary. The maximum rate of metabolism was significantly higher in naive rats (11.5 ± 0.04 mg/kg/hr) than in pregnant rats (9.36 ± 0.14 mg/kg/hr). The estimated K_m values in these two groups of rats were, respectively, 0.37 ± 0.02 and 0.50 ± 0.11 mg/liter. To obtain a consistent description of the gas uptake curves with naive female rats it was necessary to include a first-order metabolic component with a rate constant of 3.6 ± 0.2 hr^{-1} (Gargas *et al.*, 1986a). The low values of K_m indicate that TCE metabolism is flow limited in these rats (Andersen, 1981) and, as expected, K_m had relatively little impact on the simulations when set at values below about 0.5 mg/liter.

TABLE 2

KINETIC CONSTANTS FOR MODELING TRICHLOROETHYLENE AND TRICHLOROACETIC ACID IN THE NAIVE AND PREGNANT RAT

	Naive dam	Pregnant dam
Partition coefficients		
TCE		
Blood/air	15.00	13.20
Liver/blood	1.46	1.66
Rapidly perfused/blood	1.46	1.66
Slowly perfused/blood	0.46	0.52
Fat/blood	29.83	33.90
Mammary tissue/blood	—	4.57
Placenta/blood	—	0.52
Fetal blood/air	—	9.60
Fetal tissue/fetal blood	—	0.51
TCA		
Placenta/maternal blood	—	0.74
Metabolic constants		
TCE		
V_{max} (mg/hr)[a]	3.02	2.53
K_m (mg/liter)	0.25	0.25
K (hr^{-1})[b]	7.08	0.0
V_d (fetal litter, ml)	—	0.034–25.1
PO (unitless)	—	0.12
TCA		
V_d (maternal, liter)[c]	—	0.185
V_d (fetal litter, ml)	—	0.034–25.1
K (hr^{-1})[d]	—	0.076
P1 (liter/hr)	—	0.0013
P2 (liter/hr)	—	0.0020

[a] Body weight fixed at 0.175 kg, V_{max} (pregnant rat) = 9.18 · body $wt^{0.74}$ and V_{max} (naive rat) = 10.98 · body $wt.^{0.74}$
[b] Body weight fixed at 0.175 kg, $K = 4.2$/body $wt.^{0.3}$
[c] Body weight fixed at 0.175 kg, $V_d = 0.618$ · body wt.
[d] Body weight fixed at 0.175 kg, $K = 0.045$/body $wt.^{0.3}$

For the sake of consistency, the K_m value was set to 0.25 mg/liter for all groups of rats and the gas uptake results re-optimized. The new V_{max} values were 10.98 ± 0.155 and 9.18 ± 0.078 mg/kg/hr for the naive and pregnant rats, with a first-order rate constant of 4.2 ± 0.08 hr^{-1} in the naive rats. These metabolic constants were used for all subsequent simulations.

Gut uptake rate constant for TCE. Peak blood concentrations in naive female rats were obtained within 4 min after bolus intubation of 7.6 mg TCE/kg in a water solution (Fig. 3). The simulated blood time course curve was fit to experimental data by using a four-compartment physiological model (Ramsey and Andersen, 1984) and optimizing for only K_a, the first-order uptake rate constant, until a best least squares fit to the data was obtained. The resulting value for K_a, 5.4 ± 0.42 hr^{-1}, was then used in simulating the rate of gastrointestinal absorption of TCE during pregnancy.

Pharmacokinetics of intravenously administered TCA. The plasma TCA elimination after intravenous dosing with 4 mg TCA/kg

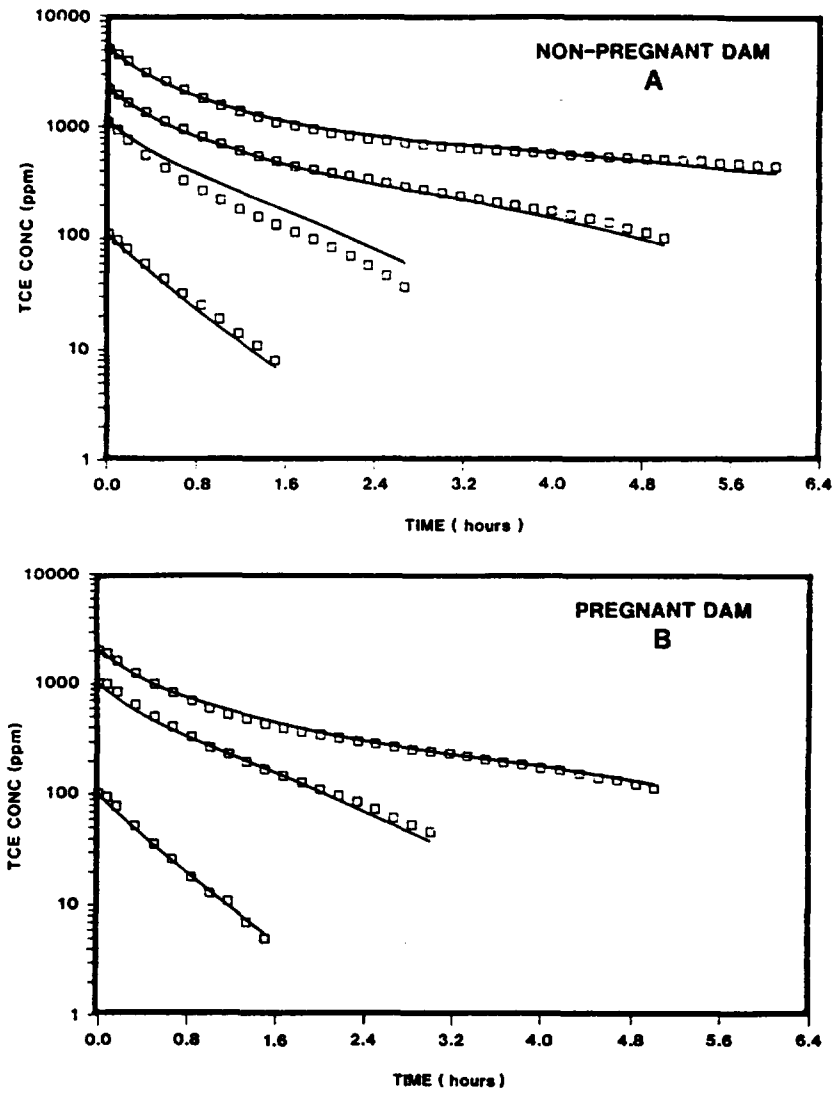

FIG. 2. The uptake of TCE from a closed recirculated atmosphere. The smooth curves were generated by the computer model and the squares represent experimentally determined atmospheric levels of TCE. (A) Naive female rats. The initial chamber TCE concentrations were 111, 1100, 2200, and 5075 ppm. Four rats were used for each exposure. (B) Pregnant rats (Day 13–15 of pregnancy). The initial chamber TCE concentrations were 103, 1005, and 2050 ppm. Four rats were used for each exposure.

in a saline solution (not shown) was adequately described by a one-compartment model with an elimination rate constant and 95% confidence interval of 0.046 (0.039–0.053) hr^{-1}. The estimated volume of distribution for TCA in the pregnant rat was 0.508 (0.444–0.675) liters/kg.

Acute 4-hr TCE inhalation exposure. The PB–PK pregnancy model was used to predict the TCE blood time course in Day 12 pregnant rats exposed to 618 ppm TCE for 4 hr (Figs. 4A, 4B). In addition to acting as a validation of the TCE portion of the simulation model (Fig. 4A), this experiment allowed an

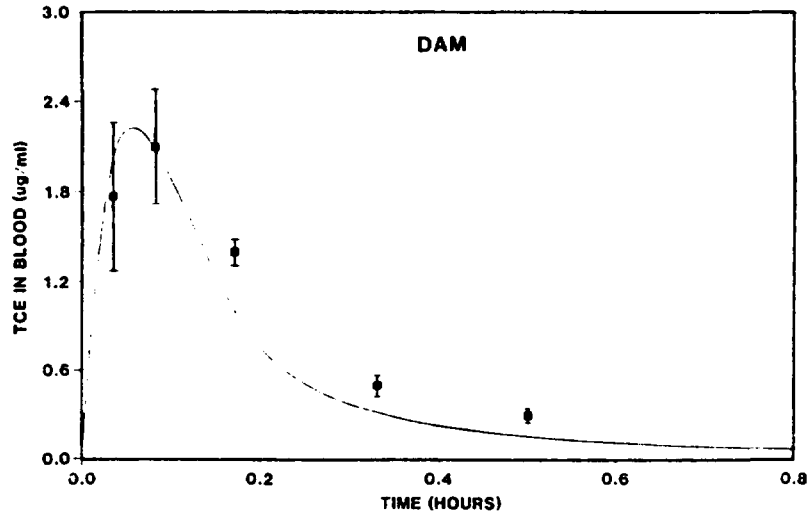

FIG. 3. Oral uptake of TCE after bolus intubation of a water solution with 7.6 mg TCE/kg. Mean TCE-blood concentrations with standard deviation bars are depicted with a smooth line generated by computer model ($n = 3$).

estimation of the yield of TCA from TCE metabolism and of the pharmacokinetic characteristics of TCA formed during the inhalation exposure. TCE oxidation produces trichloroacetaldehyde which is reduced to trichloroethanol or oxidized to TCA. The proportion of TCE oxidized to TCA, designated PO, was estimated to be 0.12 in this exposure situation (Fig. 4B). The volume of distribution for TCA was set at 0.618 liter/kg and the optimized elimination rate constant was 0.045 ± 0.0025 hr^{-1} for a 1-kg animal. These kinetic constants for TCA distribution and elimination were used for all subsequent simulations.

Subchronic TCE inhalation exposure. The acute inhalation exposure of the pregnant rat at Day 12 of gestation produced information on the kinetics of TCA in the dam. Fetal TCA exposure characteristics had to be inferred from experiments later in pregnancy when the fetuses were of sufficient size for TCE and TCA blood analyses. Consequently, the subchronic inhalation exposure was used (1) to test the fidelity of the PB–PK model both for predicting TCE concentrations in the dam and fetus (Fig. 5A) and for predicting TCA concentrations in the dam (Fig. 5B), and (2) to estimate the transfer coefficients for TCA from the maternal plasma into fetal plasma (Fig. 5B).

On the basis of subchronic inhalation results, the TCA transfer coefficients were adjusted to give an adequate representation of the fetal plasma TCA concentration at Day 20 of gestation (Fig. 5B). To produce correspondence with the data, PA1 and PA2 had to be set at different values (see Appendix II). PA1, the maternal to fetal transfer coefficient, was 0.0013 liter/hr, while the fetal to maternal coefficient was estimated to be 0.0020 liter/hr. With the estimation of these transfer coefficients, all parameters of the PB–PK pregnancy model were available and the time-courses of both TCE and TCA could be predicted for the subchronic drinking water and gavage exposure regimens. It bears emphasis that with the exception of the fitted fetal plasma TCA concentrations in the inhalation studies, all subchronic results were predicted from the PB–PK pregnancy model.

Trichloroethylene exposure during pregnancy. The experiments during pregnancy were not intended to be large scale examina-

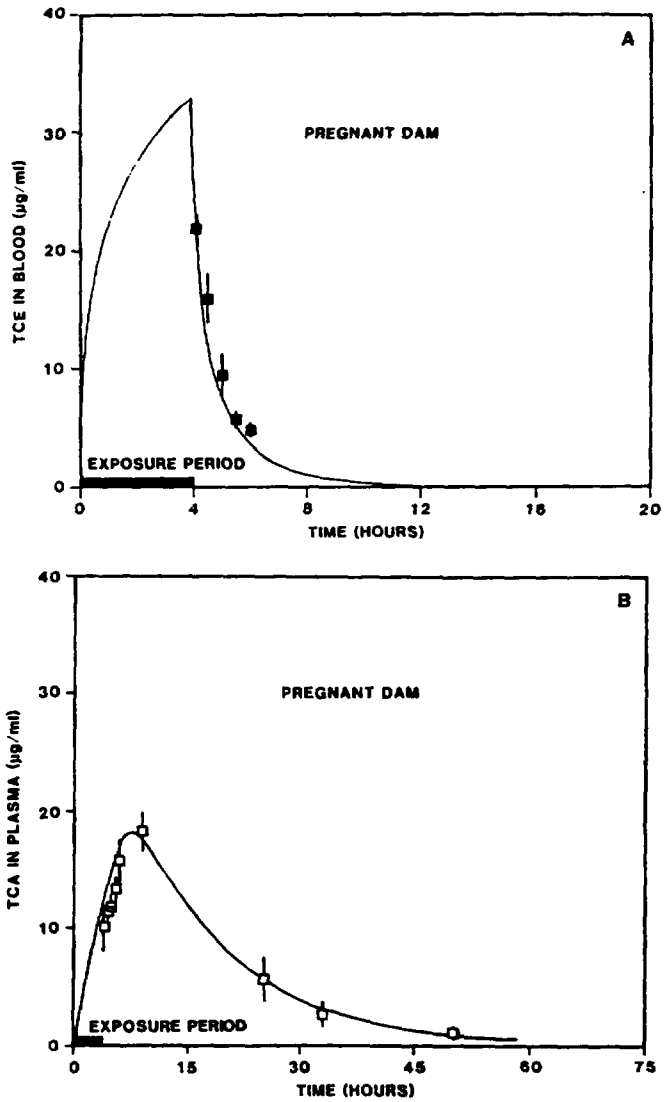

FIG. 4. Comparison of predicted (solid continuous lines) and experimental concentrations of TCE in venous blood (A) and TCA in plasma (B) of pregnant rats following a 4-hr 600.4 ppm inhalation exposure on Day 12 of gestation. Data points are means ± standard deviation ($n = 6$).

tions of the time course behavior of TCE and TCA at multiple times during gestation. The guiding philosophy in this study was to develop a PB–PK model for the pregnant rat with minimal reliance on *in vivo* experimentation and then to predict expected maternal and fetal TCE and TCA exposure from the model. The *in vivo* experiments, which were still tedious and required killing significant numbers of dams and fetuses, were purposely limited in scope and were designed to probe the ability of this modeling approach to provide a reasonable representation of the observed results with both TCE and TCA by three routes of administration at several restricted times during gestation. The maternal

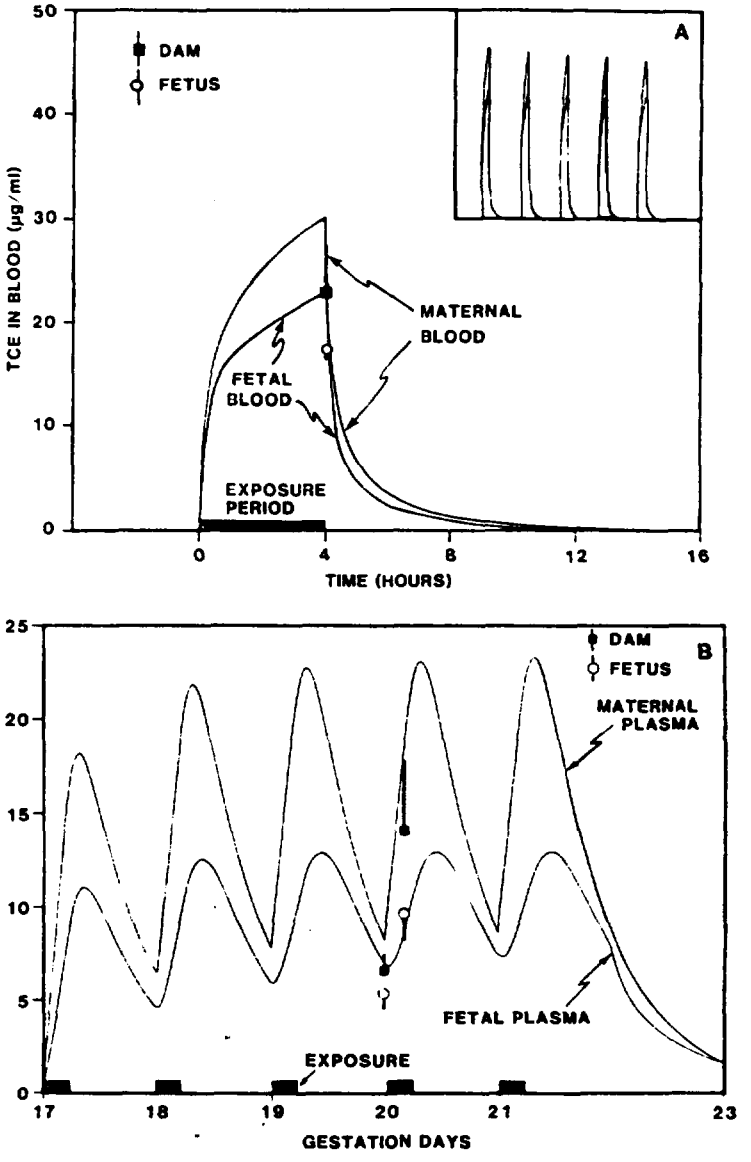

FIG. 5. Inhalation. Comparison of a portion of the predicted (solid continuous lines) and the experimental concentrations of TCE in maternal and fetal blood (A) and TCA in maternal and fetal plasma (B). Pregnant rats were exposed by inhalation to 618 ppm TCE, 4 hr/day, 5 days/week, for 3 weeks. Maternal blood (n = 3) and pooled fetal blood were collected on Day 20 of gestation, 20 hr after exposure the previous day for TCA analysis and on Day 20 of pregnancy immediately after exposure (n = 4) for TCE and TCA analyses.

...al concentrations of TCE observed for ...halation (Fig. 5A) and gavage rats (Fig. ...) compared very favorably with prediction and none were off by more than a factor of 2. The greatest discrepancy was observed with the blood TCE levels in the dams dosed by gavage. For comparison, the peak TCE concentration at the end of the 4-hr inhalation at

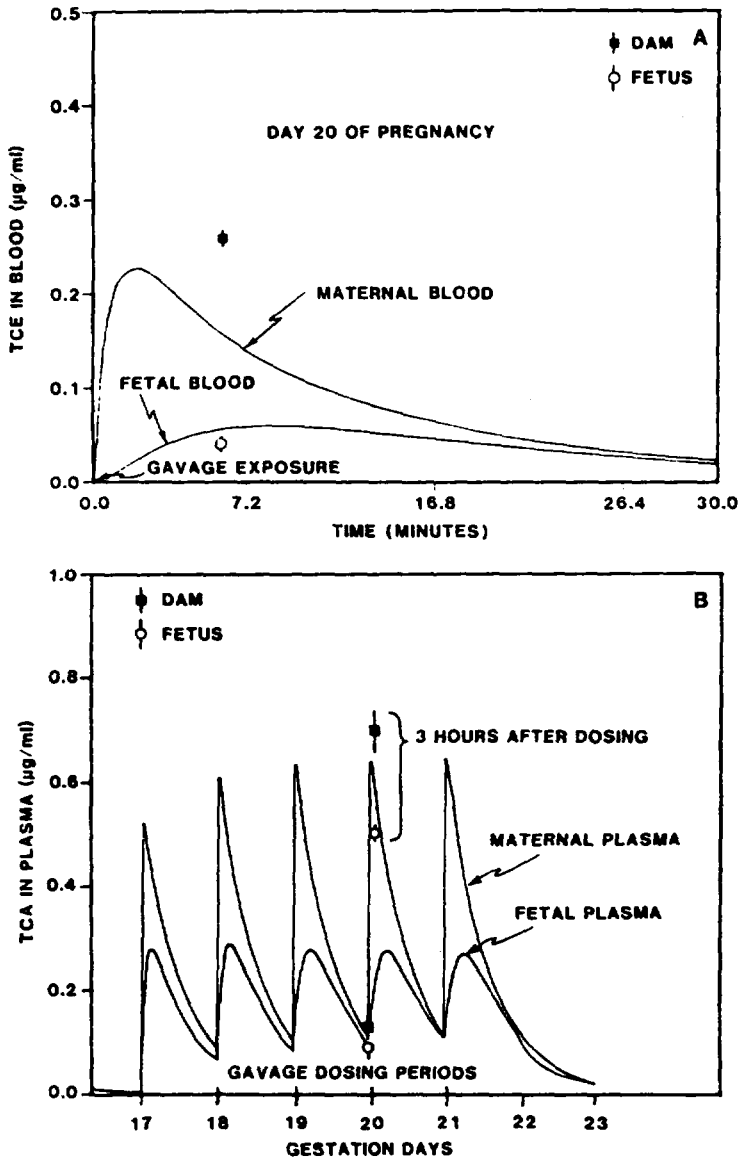

FIG. 6. Gavage. Comparison of a portion of the predicted (solid continuous lines) and the experimental concentration of TCE in maternal and fetal blood (A) and TCA in maternal and fetal plasma (B). Pregnant dams were given bolus intubation (2.3 mg TCE/kg) in water, 5 days/week, for 3 weeks. Maternal (n = 4) and pooled fetal blood was collected for TCE and TCA analyses after dosing on Day 20 of pregnancy and three hours post exposure (n = 5) on the same day for TCA analysis.

600 ppm was nearly 24 μg TCE/ml blood, while the gavaged rats had a measured concentration less than 0.3 μg TCE/ml blood at 5 min after dosing. TCE was not found at measurable concentrations in maternal or fetal blood of the rats exposed by ingestion of TCE in drinking water. Consistent with this observation, the peak TCE blood concentrations predicted from the model were below the limit of detection (0.03 μg TCE/ml blood).

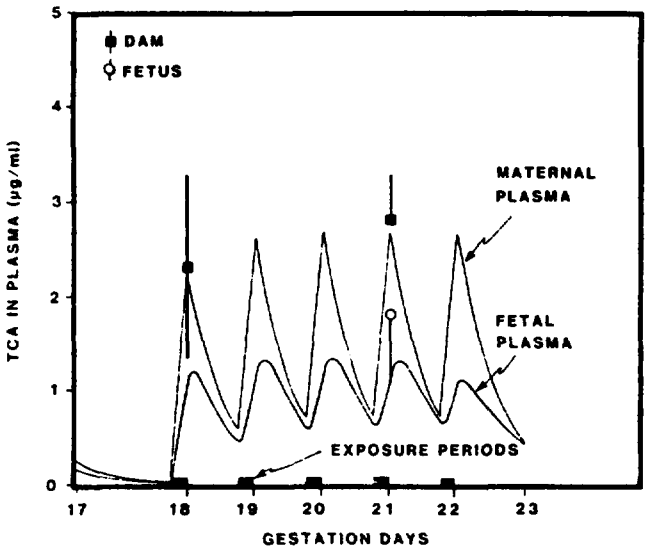

FIG. 7. Drinking water. Comparison of a portion of the predicted (solid continuous lines) and the mean experimental concentrations of TCA in maternal and fetal plasma. Rats were provided daily drinking water containing an initial mean TCE concentration of 350 μg TCE/ml water, 5 days/week, for 3 weeks. TCE-water consumption remained constant throughout pregnancy. Maternal blood (n = 4) was collected between 0900 and 1000 hr of Day 18 of pregnancy for TCA analysis. Maternal blood (n = 6) and pooled fetal blood were also collected at the same time of day on Day 21 of pregnancy for TCA and TCE analyses.

Trichloroacetic acid exposure during pregnancy. TCA concentrations in the fetuses of dams exposed by inhalation were used to adjust the transfer coefficients and do not represent predictions from the PB–PK pregnancy model. The maternal TCA plasma concentration (Fig. 5B), predicted on the basis of the parameters obtained by limited experimentation and by review of the literature regarding physiological parameters in pregnant rats, was in good agreement with the observed values. For the drinking water and single dose gavage portions of the study there was good agreement between observed and predicted TCA concentrations in the maternal plasma (Figs. 6B and 7). The model tended to slightly under predict fetal TCA concentrations. The largest discrepancy was observed with the gavage rats where the 3-hr postgavage fetal plasma TCA concentration was almost twice the predicted value (Fig. 6B). In general, there was very good agreement between the predicted and observed behavior of TCA over the three dose routes in both the dam and fetus. Maximum TCA concentrations in maternal plasma were 13, 0.7, and 2.8 μg TCA/ml plasma for the inhalation, gavage, and drinking water rats, respectively.

DISCUSSION

The V_{max} values obtained for TCE in female rats and its dependence on the physiological state of the rat were consistent with other literature. Turcan *et al.* (1980, 1981), for instance, reported that the cytochrome P-450 monooxygenase system activity is reduced during pregnancy, a decrease that has been linked to altered steroid hormones (Neims *et al.*, 1976). Our pregnant female rats had a V_{max} (9.18 mg/kg/hr) value significantly lower than the V_{max} (10.98 mg/kg/hr) in naive female rats. In addition, TCE metabolism has also been examined by gas uptake methods in male rats (Andersen *et al.*, 1987).

The male rat V_{max}, 11.0 mg/kg/hr, was slightly higher than the V_{max} in naive, female rats. On a comparative basis, TCE has a higher rate of oxidative metabolism than other chloroethylenes. For male rats the V_{max} values for vinyl chloride, vinylidene chloride, *trans*-dichloroethylene and *cis*-dichloroethylene were respectively, 2.5, 7.5, 3.0, and 3.0 mg/kg/hr (Gargas *et al.*, 1988). Similar comparative studies of the entire family of chloroethylenes have not been conducted in female rats. The techniques for PB–PK modeling in pregnancy developed in this paper could easily be expanded for application with these other ethylenes, as well as a variety of other volatile chemicals, once a suitable data base is established for female tissue partition coefficients and metabolic rates for these other chemicals.

Determining the exposure profile of the developing fetus to xenobiotics as a result of maternal xenobiotic exposure is of considerable toxicological interest for teratology and reproductive studies. Using the rat as a model, physiologically based mathematical models have been developed for evaluating the disposition of drugs in the pregnant dam and developing fetus. Olanoff and Anderson (1980) constructed a time-dependent physiological model for pregnancy. Seven maternal compartments and seven fetal compartments were used to describe the kinetics of tetracycline in the pregnant rat. Temporal changes in maternal and fetal blood flows to the placenta and growth of the placenta and fetal compartments were described as gestation progressed. In other work, Gabrielsson and Paalzow (1983) developed a physiologically based pregnancy model for morphine using six maternal compartments and one fetal compartment. More recently Gabrielsson *et al.* (1985) developed a physiologically based pregnancy model for methadone in the pregnant rat using 11 maternal compartments and one fetal compartment. Our PB–PK pregnancy model was developed to describe the uptake, disposition and elimination of TCE, a well metabolized, volatile chemical, as well as TCA, one of its nonvolatile metabolites.

The acute and subchronic TCE inhalation studies both served useful purposes in developing the compartmental model for TCA in the pregnant rat. The acute 4-hr inhalation study provided information for determining the amount of oxidized TCE that is converted to TCA and for refining the compartmental kinetic constants (K and V_d) for TCA in the dam. The estimate of the proportion of TCE converted to TCA (12%) is consistent with other literature. DeKant *et al.* (1986) reported that about 17% of a 2 mg/kg dose of TCE was excreted in the urine as TCA in adult female Wistar rats. Ogata *et al.* (1979) reported that female Wistar rats excreted a 2.3:1 ratio of trichloroethanol to TCA, which is about 30% TCA if all the metabolized TCE is accounted for by the alcohol and acid.

The clearance term, PA1 (Fig. 1), was 0.0013 liter/hr. PA2 (Fig. 1), a composite rate constant consisting of transfer rate and tissue solubility information was given a value of 0.0020 liter/hr (see Appendix II).

The actual litter size for the dams ranged from 3 to 12 pups per litter, and the average was 7. To better understand the effect of litter size on the disposition of TCE and TCA in the dam and fetus, the pregnancy model was exercised assuming 1, 3, or 12 fetuses per dam. This was done by adjusting fetal litter growth (Eq. 1) and placental growth (Eq. 2) to reflect the appropriate number of fetuses. Total blood flow to the placenta for the whole litter was still assumed to be 25% of adjusted maternal placental blood flow. TCA placental transfer coefficients, PA1, PA2, were adjusted, accordingly (e.g., PA1/7 equals PA1 for litter size equal to 1). No appreciable changes were observed in simulated dam or fetus TCE-blood or TCA-plasma time courses as a result of adjusting litter size within the model structure.

There are several advantages in conducting inhalation exposures to develop physiological models with volatile organics and their metabolites for multiple routes of exposure.

Inhalation exposure provides a means for producing a substantial chemical body burden which facilitates examination of tissue concentration time course data. Our inhalation exposures with the pregnant rats provided estimates of the kinetic constants for TCA and the yield of TCA from oxidative metabolism of TCE. Equally important, the TCE blood concentration time course could be predicted over a fairly wide range as TCE was taken up and cleared from the body. These results set the stage for the other exposure routes. The modeling results of the subchronic drinking study were very promising. Although individual drinking patterns of the rats were greatly simplified (zero order rate of intake, 1200 to 0600 hours), the stable metabolite, TCA, was detected in the dam and fetal plasma near model predicted plasma concentrations (Fig. 7). Modeling results of gavage dosing were also promising for TCE, and only slightly less successful for TCA. Peak TCE blood levels after a single gavage dose (7.6 mg TCE in water/kg) occurred within a similar time period (4 min) as reported by other investigators. D'Souza *et al.* (1985) reported that peak TCE blood concentrations occurred within 6 to 10 min after using gavage dosing with PEG 400 as vehicle. Withey *et al.* (1983) reported that peak TCE blood levels occurred within 5 to 6 min after gavage dosing using water as vehicle. Using a first-order rate constant of 5.4 hr^{-1} to describe the gastrointestinal absorption of TCE (Fig. 3), the model adequately described the uptake and clearance of TCE from the blood after a bolus gavage dose. For the repeated gavage dosing, the maternal and fetal blood TCE levels were in close agreement with the model predictions (Fig. 6A). However, TCA in the fetal plasma was not detected in two of four litters whose mothers had just been dosed by gavage (Fig. 6B), and 3 hr after dosing the model underpredicted the fetal plasma levels by a factor of 2.

The pregnancy model had several simplifying assumptions. For example, the fetal and placenta compartments were described only from Days 14 of gestation, and there was no fetal metabolism of TCE. Fetuses have a limited P450 metabolic capacity near term (Neims *et al.*, 1976). The fetal compartment was described as just one compartment for both the TCE and TCA disposition. Despite these simplifications, the model attempted to account for the major physiologic, biochemical, and physical-chemical processes which govern the disposition of TCE and TCA in the pregnant rat and generally good agreement was observed between predicted and measured blood TCE and plasma TCA concentrations (Figs. 5–7). Withey and Karpinski (1985) exposed pregnant Sprague–Dawley rats (Day 17 of pregnancy) for 5 hr to several chlorinated hydrocarbon vapors. Maternal blood and fetal tissue were collected immediately following exposure for hydrocarbon analyses. To describe these data within our PB–PK pregnancy model, body weights were set to those reported by Withey and Karpinski (1985) and simulations were conducted for exposures at 302, 1040, 1559, and 2088 ppm TCE. The model was configured to predict maternal blood and fetal tissue concentrations at 5 min postexposure. To obtain an optimal fit with these Sprague–Dawley rats, V_{max} was increased to 12.0 mg/kg/hr and blood:air PC was reduced to 10.0. These constants compare to the respective values of 9.2 mg/kg/hr and 13.2 in the Fischer rats. The overall correspondence between the observed and predicted data with only minor changes in model parameters was very good (Fig. 8), increasing our confidence in the applicability of this PB–PK approach for analyzing the disposition of TCE in pregnant rats under a variety of exposure conditions.

One use of the physiologically based pregnancy model is to compare TCE and TCA exposure profiles of the dam with the exposure profiles of the fetus as a result of maternal exposure to TCE. In addition, route of exposure comparisons can also be conducted. One measure of chemical exposure is the cumulative blood-TCE or plasma-TCA con-

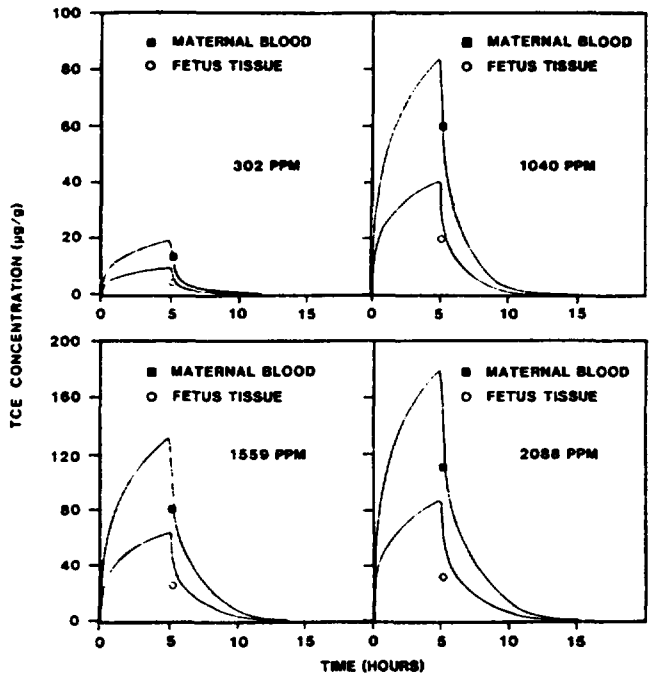

FIG. 8. PB–PK model predicted and experimentally determined TCE levels in maternal blood and fetal tissue directly after 5-hr inhalation exposures at 302, 1040, 1559, and 2088 ppm TCE. Experimental data were taken from Withey and Karpinski (1985). V_{max} was set to 12.0 mg/kg/hr and the blood/air PC to 10.0.

centration area under the curve (AUC) for the dam and fetus. The AUC blood or plasma value provides a measure of the availability of the TCE or TCA for transport to various target tissues. Table 3 shows the model derived AUC blood-TCE and plasma-TCA values for the fetus and dam during gestation. Table 3 also includes predicted amounts of TCE metabolized and exhaled for the three routes of exposure. The fetal blood AUC values for TCE were 67, 69, and 76% of the maternal venous blood AUC values for the gavage, drinking water, and inhalation animals, respectively, for Days 14 to 22 of gestation. The fetal plasma AUC values for TCA were 64, 63, and 64% of the maternal AUC values for the gavage, drinking water and inhalation animals, respectively, for Days 14 to 22 of gestation (Table 3). Thus, the fetus should be susceptible to both TCE and TCA insult as a result of the maternal TCE exposure. While TCE is not a potent rodent teratogen, subtle biochemical or neurological affects may occur in the developing fetus as a consequence of maternal exposure to TCE. Recently Taylor *et al.* (1985) has shown irreversible behavioral deficits (locomotor activity) in rat pups indirectly exposed to TCE and its metabolic by-products from maternal exposure to TCE in the drinking water. In the drinking water exposure the predominant tissue exposure was from TCA (585 vs 2.7 (mg-hr)/liter). By inhalation the AUC for TCE is greatly increased to 1864 (mg-hr)/liter, with a 10-fold increase in TCA exposure (585 vs 5853 (mg-hr)/liter). It would be interesting to examine the *in utero* toxicity of TCE by inhalation exposures.

The use of PB–PK pregnancy models for examining fetal dosimetry is important for establishing more rigorous dose–response relationships for these behavioral teratology

TABLE 3

MODEL DERIVED EXPOSURE ESTIMATES FOR TCE AND TCA DURING 3 WEEKS OF PREGNANCY

	Route of exposure		
	Gavage	Drinking water	Inhalation
Dam			
TCE metabolized (mg)	5.30	32.81	311.97
TCE exhaled (mg)	0.24	1.01	873.02
TCE in maternal blood (AUC) (mg/hr/liter)	0.72 (0.30)[a]	2.69 (1.05)[a]	1864.05 (725.00)[a]
TCA in maternal plasma (AUC)	106.43 (44.84)[a]	584.52 (261.57)[a]	5201.20 (2298.79)[a]
Fetus			
TCE in fetal blood (AUC)	0.20	0.72	552.35
TCA in fetal plasma (AUC)	28.95	164.10	1473.66

[a] AUC value in parentheses corresponds to modelled fetal development period (Day 14 to the end of Day 22 of pregnancy). This AUC value was used to compare the maternal exposure to TCE and TCA with fetal exposure to these chemicals.

studies. With further validation of fetal exposure, PB–PK modeling may provide an important framework for interpreting a variety of teratology and reproductive toxicology experiments.

APPENDIX I: PREGNANCY MODEL NOMENCLATURE

TCE

- P Maternal blood/air partition coefficient (liter blood/liter air)
- P_1 Fetal blood/air partition coefficient (liter blood/liter air)
- P_i Tissue (i)/blood partition coefficient (liter blood/liter tissue)
- Q_i Blood flow to ith tissue (liter/hr)
- C_a Arterial blood concentration (mg/liter)
- C_i Concentration in ith tissue (mg/liter)
- C_{vi} Venous blood concentration leaving ith tissue (mg/liter)
- A_i Amount in ith tissue (mg)

TCA

- A_{TCAi} Amount in ith tissue (mg)
- V_d Volume of distribution (liter)
- C_{TCAi} Concentration in ith tissue (mg/liter)
- K Elimination rate constant (/hr)
- PI_i Tissue (i)/blood partition coefficient (liter blood/liter tissue)

Subscripts i

TCE and TCA

- pla placenta
- fet fetus
- p plasma
- l liver

APPENDIX II: MODEL COMPARTMENTS

TCE Equations. The tissue mass balance equations for TCE were developed as outlined by Ramsey and Andersen (1984), with slight elaboration to include both saturable and first-order metabolic processes in the liver (Gargas *et al.*, 1988).

The rate of change in the amount of TCE in the placenta consists of two terms, one re-

lated to the maternal circulation to the placenta and the other, to the fetal circulation to the placenta

$$dA_{pla}/dt = Q_{pla}(C_a - C_{pla}/P_{pla}) - dA_{fet}/dt. \quad (4)$$

The second term is identical to the rate of change of TCE in the fetus

$$dA_{fet}/dt = Q_{fet}(C_{pla}/P_{pla} \cdot P_t/P - C_{fet}/P_{fet}). \quad (5)$$

The TCE concentration in the fetal drainage leaving the placenta includes a ratio of partition coefficients accounting for differences in partitioning of TCE between maternal and fetal blood (Table 2).

TCA equations. The rate of TCA production for the pregnant rat is expressed as a proportion (PO) of the rate of TCE metabolism (PO = 0.12). A stoichometric conversion factor (SC equals 163.4/131.4) accounts for the molecular weight increase which occurs as a result of the enzymatic conversion of TCE to the oxidized TCA metabolite

$$dA_{TCA}/dt = (PO)\frac{V_{max} \cdot C_{vl}}{K_m + C_{vl}}(SC). \quad (6)$$

The rate of change in the amount of TCA in the maternal plasma is described by the TCA production term (Eq. (6)), a first-order plasma elimination term, and a term describing the flow of blood to the placenta

$$dA_{TCAp}/dt = dA_{TCA}/dt - V_d \cdot K \cdot C_{TCAp} - Q_{pla}(C_{TCAp} - C_{TCApla}/PI_{pla}). \quad (7)$$

The concentration of TCA in the maternal plasma is

$$C_{TCAp} = A_{TCAp}/V_d, \quad (8)$$

and A_{TCAp} is the integral of Eq. (7):

$$A_{TCAp} = \int_0^t dA_{TCAp}. \quad (9)$$

In the placenta, TCA was considered to be flow-limited with respect to the maternal blood supply, but transplacental movement was modeled as a diffusion process. The equation

$$dA_{TCApla}/dt = Q_{pla}(C_{TCAp} - C_{TCApla}/PI_{pla}) - PA1 \cdot C_{TCApla}/PI_{pla} + PA1 \cdot C_{TCAfet}/PI_{fet}, \quad (10)$$

contains placenta/maternal blood (PI_{pla}) and fetal tissue/maternal blood (PI_{fet}) partition coefficients. PI_{pla} was estimated by the ratio of the placenta/saline and the maternal blood/saline partition coefficients. No estimate of PI_{fet} was obtained in these present studies. Thus the equation used for placental TCA was simplified to

$$dA_{TCApla}/dt = Q_{pla}(C_{TCAp} - C_{TCApla}/PI_{pla}) - PA1 \cdot C_{TCApla}/PI_{pla} + PA2 \cdot C_{TCAfet}. \quad (11)$$

PA2 then has information on the permeation coefficient and tissue solubility. For fitting the fetal TCA concentrations, PA1 and PA2 were estimated as independent parameters.

The last two terms in Eq. (11) are for describing the rate of change in the amount of TCA in the fetus. The concentration in the fetus is the integration of these terms divided by the calculated fetal volume.

Exposures. Three routes of TCE exposure were considered; gavage, drinking water, and inhalation. The gavage TCE exposure is incorporated into the pregnancy model by assuming that the amount of TCE absorbed from the gastrointestinal (GI) tract into liver of the dam is first order:

$$dA_{abs}/dt = K_a \cdot A_{gi}. \quad (12)$$

The amount of TCE remaining in the maternal GI tract (A_{gi}) is:

$$A_{gi} = (A_{go})\exp[-K_a(t)]. \quad (13)$$

where A_{go} equals the amount of TCE (mg) administered per gavage, K_a equals 5.4/hr^{-1}, and time t is $0 \leq t \leq 24$ hr.

The drinking water TCE exposure is described by assuming that rats drink the TCE water solution for 6 hr at a zero-order rate

(K_{dw} equals 2.42 ml/hr) from 2400 to 0600. The rate of change in the amount of TCE orally ingested by drinking TCE in water is

$$dA_{dw}/dt = K_{dw} \cdot C_{dw}. \quad (14)$$

Because of the nonspecific loss of TCE from the water in the bottles, a first-order loss rate (k_s) was determined (0.031/hr, $t_{\frac{1}{2}}$ = 22.4 hr). TCE concentration in the drinking water bottle is:

$$C_{dw} = (C_{dwi})\exp[-k_s(t)]. \quad (15)$$

C_{dwi} is the initial concentration of TCE in the drinking water bottle (mg/liter) and time, t is $0 \leq t \leq 24$ hr. The zero-order drinking rate is incorporated into the model by adding the input rate equation to the liver compartment of the dam.

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